REMARKS

In the claims

Claims 1–3, 7 and 10–20 are currently under examination with claims 4–6, 8, 9 and 21–23 withdrawn due to restriction/election. Claims 23 and 24 are added by this paper. The allowability of claim 3 is acknowledged.

Finality

In the Office Action of August 9, 2006, a new anticipation rejection was presented in view of the newly cited Pierschbacher et al. (WO 91/15515) reference. Contrary to the assertion in the Office Action, this rejection was not necessitated by Applicants' amendments. Original claim 1 and amended claim 1 both defined the compound as being of formula I which is a cyclic formula. Applicants courteously request the finality of the Office Action be withdrawn. See, M.P.E.P. §706.07.

Rejection under 35 U.S.C. §112, first paragraph (enablement)

At pages 4–5, the Office Action alleges that the specification fails to comply with the enablement requirement. It is alleged that the specification does not provide a clear definition of the term "one or more" α-aminocarboxylic acid residues, which comprise the R^1 group of the claimed polypeptide. Applicants respectfully disagree with this contention.

Contrary to the Office's allegation, Applicants submit that the specification provides clear definition of the amino acid residues which make up the peptide compounds claimed by the instant invention. For example, at page 4, lines 28–39 of the instant specification, it is expressly stated that:

> Particularly active compounds are those of the formula I in which an octapeptide sequence cyclo(Arg-X^1-Asp-X^2- X^3-X^4-X^5-X^6), in which the radicals X^1, X^2, X^3, X^4, X^5, X^6 have the meanings indicated, is expanded by R^1.The effect of the ring expansion is shown in Fig. 1 by the example of cyclo(Arg-Gly-Asp-Leu-D-Asp-Ala-Leu-Arg)

> [Emd 271588]. Ac-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-NH_2 [EMD 271293] serves as a comparison compound.
>
> Some cycol(Arg-Gly-Asp-Leu-D-Asp-Ala-Leu-Arg-R^1) peptides, the distance from R^1 (calculated) and the value of Q (IC_{50} [substance]/ IC_{50} [EMD 271293]) and -log Q are given in the following table. (Emphasis added)

And the disclosure at page 5 which states

> The graphic representation can be seen in Fig. 1. Particularly active compounds are obtained if the spacer length R^1 has reached approximately 500 pm.
>
> Particularly preferred compounds of the formula I are those in which R^1 is one or more ω-amino carboxylic acid residue(s), the ω-amino carboxylic acid residue(s) having a length of 600 to 2500 pm...very particularly preferable those having a spacer length of 600 to 2500 pm. (Emphasis added)

Accordingly, it is clear and fully supported by the disclosure provided by the Applicants' specification that R^1 is defined by two characteristics, namely

(a) it is comprised of one or more α-amino carboxylic acid residue(s) or ω-amino carboxylic acid residue(s)

(b) the combined α-amino carboxylic acid residue(s) or ω-amino carboxylic acid residue(s) (and thus R^1) has a length of 500–2500 pm

Furthermore, Applicants submit that the claimed length and/or the composition of the substituent R^1 (one or more α-aminocarboxylic acid residue(s) having a length of 500–2500 pm) in the peptide compounds claimed herein is fully commensurate with the a glycine residue with an average length of 370 pm, for example, when R^1 comprises two glycine residues (combined length of at least 740 pm).

It is therefore courteously submitted that the claims in their current form, in view of the detailed disclosure contained in the specification, provides more than sufficient guidance to objectively enable one of ordinary skill in the art to make and use the claimed invention with an effort that is routine with in the art. Withdrawal of the rejection under 35 U.S.C. §112, first paragraph, is respectfully requested.

Rejection under 35 U.S.C. §112, first paragraph (written description)

The Office's contention that the specification lacks adequate guidance regarding the preparation and/or use of *salts and solvates* has been maintained. For example it is alleged that the "there is inadequate support in the specification for the preparation of salts and solvates." Contrary to the Examiner's assertion, we find that the specification clearly conveys possession of the concept of pharmaceutically acceptable salts and solvates. See, for example, page 1, lines 24–25 and the paragraph bridging pages 16-17. The rejection fails to present any rationale as to why such disclosure does not reasonably convey possession of the claimed subject matter.

Moreover, contrary to the implication in the rejection, the pharmaceutical art clearly does recognize the formation of *salts and solvates* of pharmaceutically active substances such as those claimed by the instant invention. For example, starting from a peptide compound, one of ordinary skill in the art would be able to employ the teaching of Vippagunta et al. and others to perform routine experimentation and successfully arrive at the claimed salts and solvates. Furthermore, it is well-established that the production of salts and solvates of existing compounds is both a routine and a trivial task for one of ordinary skill, provided that he/she is equipped with necessary compounds (solvents, acids etc.) and conditions (pH, temperature etc.) for carrying out such a process. Representative examples of such methods are amply provided throughout the specification. For example, see lines 22-40 at page 16; lines 1-25 at page 17; and Example 1. Also see the table at page 25. The Examiner is also courteously requested to review the enclosed reference on solublization and storage of synthetic peptides.

In light of the detailed disclosure in the specification and what was known to a skilled worker on or before the filing date of the instant application, it is respectfully submitted that the specification more than reasonably conveys to one of ordinary skill in the art that Applicants had possession of the claimed subject matter. To maintain the rejection under these circumstances is grossly improper and therefore the Office is courteously requested to withdraw the pending rejection.

Rejection Under 35 U.S.C. § 102 (b)

Claims 1, 2, 7, and 10–20 stand rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by the newly cited reference of Pierschbacher et al. (WO91/15515). This rejection is respectfully traversed.

It is alleged that the optional derivatization of the peptide compounds claimed herein allows for Pierschbacher's peptides to "read on" Applicants claimed invention. Applicants courteously disagree with this contention. According to standard chemical handbook (For example, Roempp's Dictionary), the terms "derivative," "derivatized" or "derivatization" mean that in an existing structure, such as a molecule, a functional group is altered by a reaction with a reagent whereas the "backbone" of the existing structure that carries said functional group remains unchanged. This definition is consistent with the description in the specification regarding the process of derivatization and the derivatives that are generated via such a process. For example, at page 11, lines 15–25 it is expressly stated:

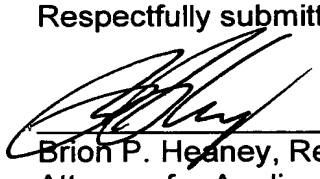
> The amino acids and the amino acid residues mentioned, such as, for example, the NH functions or the terminal amide functions, can also be derivatized, the N-methyl, N-ethyl, N-propyl, N-benzyl, or C_α-methyl derivatives being preferred. Derivatives which are additionally preferred are those of Asp and Glu, in particular the methyl, ethyl, propyl, butyl, tert-butyl, neopentyl or benzyl esters of the side chain carboxyl groups and in addition also derivatives of Arg, which can be substituted on the –NH-C(=NH)-NH_2 group by an acetyl, benzoyl, methoxycarbonyl or ethoxycarbonyl radical. (Emphasis added)

The Office Action does not provide any support for the definition of derivatives associated in the rejection. Applicants courteously submit that for Pierschbacher's peptides to anticipate the peptide compounds claimed herein, one would need to "homologize" the individual amino acid residues or start with structurally-unrelated amino acid residues. As one of ordinary skill in the art can readily attest to, the process of "homologization" is very different from the process of "derivatization" as recited in the instant specification. See, the enclosed reference on homologization and compare with the aforementioned process of "derivatization." As such, it is courteously submitted that the cited reference of Pierschbacher does not anticipate the peptide compounds claimed herein. Withdrawal of the rejection is respectfully requested.

In view of the above remarks, favorable reconsideration is courteously requested. If there are any remaining issues which could be expedited by a telephone conference, the Examiner is courteously invited to telephone counsel at the number indicated below.

No fees are believed to be due with this response; however, the Commissioner is hereby authorized to charge any fees associated with this response to Deposit Account No. 13-3402.

Respectfully submitted,



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Attorney Docket No.: **MERCK-2360**

Date: December 11, 2006

Encl:

(1) Sigma-Aldrich Brochure on dissolution and storage of synthetic peptides

(2) Wikipedia reference on Homologous series and Homologization

Homologous series

From Wikipedia, the free encyclopedia

In chemistry, a **homologous series** is a series of organic compounds with a similar general formula, possessing similar chemical properties due to the presence of the same functional group, and shows a gradation in physical properties as a result of increase in molecular size and mass (see relative molecular mass). For example, ethane has a higher boiling point than methane since it has more Van der Waals forces with neighbouring molecules. This is due to the increase in the number of atoms making up the molecule. Organic compounds in the same homologous series vary by a CH_2.

Alkanes (paraffins), alkenes (olefins), Methoxyethane (ethers), and alkynes (acetylenes) form such series in which members differ in mass by 14, 12, and 10 atomic mass units, respectively. For example, the alkane homologous series begins with methane (CH_4), ethane (C_2H_6), propane (C_3H_8), butane (C_4H_{10}), and pentane (C_5H_{12}), each member differing from the previous one by a CH_2 group (or 14 atomic mass units).

Similarly, there is the alcohol homologous series that starts with methanol (CH_4O), ethanol (C_2H_6O), as primary alcohols, isopropanol (C_3H_8O) as a simple secondary alcohol, and a simple tertiary alcohol is tert-butanol ($C_4H_{10}O$).

Even while the *general formula* are the same, they have different structures that can lead the exact same compound to different properties, although they will always present the same chemical properties while as a homologous compound.

Compounds in each set have the same little group of atoms called the functional group. Most **chemical properties** of organic compounds are due to the presence of the functional group.

Homologous series	General formula	Example	Functional group
Alkanes	C_nH_{2n+2} (n more than or equal to 1)	CH_4, $n = 1$	
Alkenes	C_nH_{2n} (n more than or equal to 2)	C_2H_4, $n = 2$	C = C
Alkynes	C_nH_{2n-2} (n more than or equal to 2)	C_2H_2, $n = 2$	C ≡ C
Alcohols	$C_nH_{2n+2}O$ (n more than or equal to 1)	CH_4O, $n = 1$	- OH
Carboxylic acids	$C_nH_{2n}O_2$ (n more than or equal to 1)	CH_2O_2, $n = 1$	- COOH

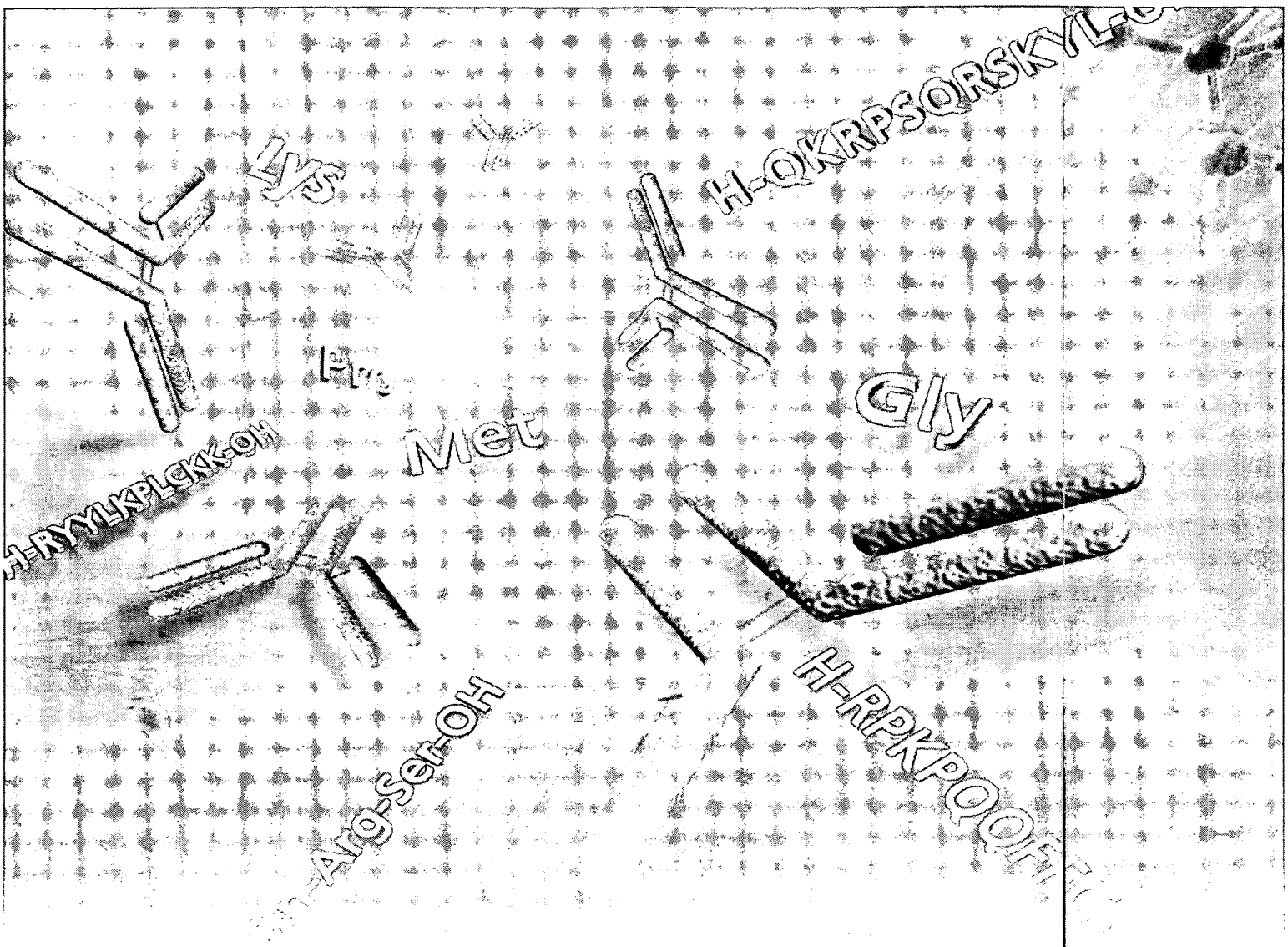
Where n represents the number of carbon atoms present.

Homologization

Homologization is any chemical process converting one member of a homologues series to the next. For example the reaction of aldehydes and ketones with diazomethane or methoxymethylenetriphenylphosphine effectively inserts a methylene unit in the hydrocarbon chain and the reaction product is the next homologue. Chain homologization in sugars occurs in the Kiliani-Fischer synthesis.

Retrieved from "http://en.wikipedia.org/wiki/Homologous_series"

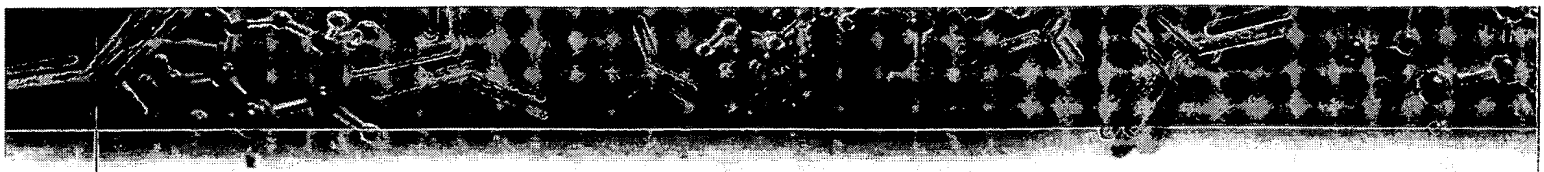
Category: Organic chemistry



Storage and Handling Synthetic Peptides

GUIDELINES

SIGMA GENOSYS



Handling and Storage of Synthetic Peptides

Often times the most challenging aspect of working with synthetic peptides is determining the best solvent in which the peptide will dissolve. This document should serve as a general guide for proper storage, handling and dissolving of your synthetic peptide.

Storage Guidelines for Lyophilized Peptides

Upon receiving the lyophilized peptide, store at 4°C or colder and away from bright light. Lyophilized peptides are stable at room temperature for days to weeks, but for longer-term storage, it is safer to store at -20°C or colder.

Exposure to moisture will greatly decrease long-term stability of lyophilized peptides. Before using the peptide, remove from cold storage and allow the peptide to equilibrate to room temperature before removing the lid of the container, in order to reduce the uptake of moisture that is present in the surrounding atmosphere.

A Strategy for Dissolving Single Peptides

There is no universal solvent for solubilizing all lyophilized peptides, while also maintaining their integrity and compatibility in biological assays. Different solvents may need to be tested until an appropriate solvent is found. Selecting the best solvent for your particular peptide may be a result of a "trial-and-error" process. Wherever applicable, it is advisable to first try solvents that are relatively easy to remove by lyophilization, in case the initial solvent does not work. Therefore, it is necessary to test a portion of the peptide first before dissolving the entire peptide sample. The steps below provide a general guide for peptide solubilization.

Determining Solubility Characteristics

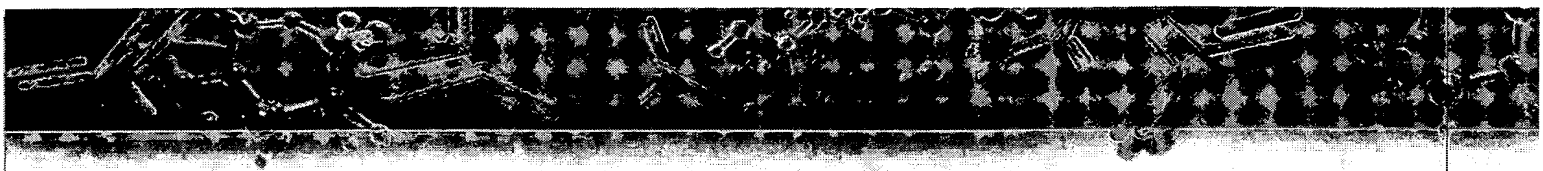
Before adding any solvent to the lyophilized peptide, it is important to evaluate the amino acid composition of the peptide as a preliminary tool in understanding the solubility characteristics of your peptide. The number and types of ionic charges in the peptide determine its solubility in aqueous solutions. In general, the more charged residues the peptide possesses, the more soluble it is in aqueous solutions. In addition, peptides generally have more charges at pH 6-8 than at pH 2-6. It is for this reason that peptides are better dissolved at near neutral pH. Among the many exceptions to the rule are peptide sequences that are very hydrophobic and those that tend to aggregate. While the hydrophobicity of the sequence is the primary cause of aggregation, peptides can also aggregate or "gel" through extensive hydrogen bonding network. The guidelines below are used to determine if the peptide is basic, acidic or neutral.

1. Assign a value of -1 to each acidic residue (D, E, and C-terminal COOH)
2. Assign a value of +1 to each basic residue (K, R and the N-terminal NH_2)
3. Assign a value of +1 to each H residue at pH<6 and zero at pH >6.
4. Count the total number of charges of the peptide at pH 7 (all D, E, K, R, C-terminal COOH, and C-terminal NH2).
5. Calculate the overall net charge of the peptide.

Dissolving Approach for Charged Peptides

Based on the above guidelines, proceed to test the solubility of the peptide using the following strategies:

1. If the overall net charge of the peptide is negative, the peptide is considered acidic. If the peptide is acidic, and/or if the total number of charges of the peptide at pH 7 is greater than 25% of the total number of residues, add a small amount of 0.1M ammonium bicarbonate to dissolve the peptide and dilute it with water to the desired concentration. Make certain that the resulting pH of the peptide solution is about 7 and adjust the pH as needed.
2. If the overall net charge of the peptide is positive, the peptide is considered basic. If the peptide is basic and the total number of charges of the peptide at pH 7 is between 10-25% of the total number of residues, add a small amount of 25% acetic acid to dissolve the peptide and dilute it with water to the desired concentration.
3. If the overall net charge of the peptide is zero, the peptide is considered neutral. If the total number of charges is greater than 25% of the total number of residues, use the strategy described in section 1. If the total number of charges is between 10-25% of the total number of residues, use organic solvents as recommended elsewhere in this document.
4. If the total number of charges of the peptide is less than 10% of the total number of residues, the use of organic solvents is recommended.



For any solvent used, the maximum concentration of the initial solvent will depend on the tolerance of your assay against that particular solvent. Before trying stronger solvents, it is necessary to sonicate the peptide solution to confirm that the peptide is insoluble in the solvent. Sonication enhances solubilization, breaking the solid peptide into smaller particles. If, after sonication, the solution has gelled, appears cloudy, or has visible particulates, the peptide has not dissolved completely but is suspended. At this point, a stronger solvent is necessary. If the peptide does not dissolve, lyophilize and remove the volatile buffer solution. Once the sample is dry, alternative solvents can be tried on the same sample.

Dissolving Approach for Hydrophobic/Uncharged Peptides

The above recommendations based on the charged nature of the peptide will likely be inadequate for dissolving peptides containing more than 50% hydrophobic residues in their sequence, neutral peptides with less than 25% charges, and/or peptides that has less than 10% charges. Under these conditions, the use of organic solvents is recommended, such as acetonitrile (ACN), dimethylsulfoxide (DMSO), or dimethylformamide (DMF). Addition of chaotropic compounds such as guanidine hydrochloride or urea can facilitate in breaking up hydrophobic interactions or reduce the "gelling" of peptides by disrupting hydrogen bonding network. Again, the concentration of the initial organic solvent or chaotropic reagents will be dependent on the tolerance of your assay system. Note also that peptide sequences containing Cys (C) and Met (M) are unstable in DMSO.

It is important to dissolve the peptide completely in the initial solvent (such as acetic acid, acetonitrile, DMSO or DMF) because the rate of dissolution of the peptides into these solvents is usually higher than in a water/solvent mixture. If the water/solvent mixture is used first to dissolve the peptide, you may end up adding a much larger than necessary amount of nonaqueous solvent to your peptide sample. Sonication may also be necessary to facilitate complete dissolution of the peptide.

After the peptide is dissolved in the initial solvent, especially those dissolved in organic solvents, dilute the peptide by slowly adding (dropwise) the peptide solution into the buffered solution with gentle but constant agitation. This is to prevent localized concentration of the peptide in the aqueous solution, which can potentially result in precipitation of the peptide. The added benefit of this strategy is that the possibility of precipitation can be visually monitored and acted upon accordingly.

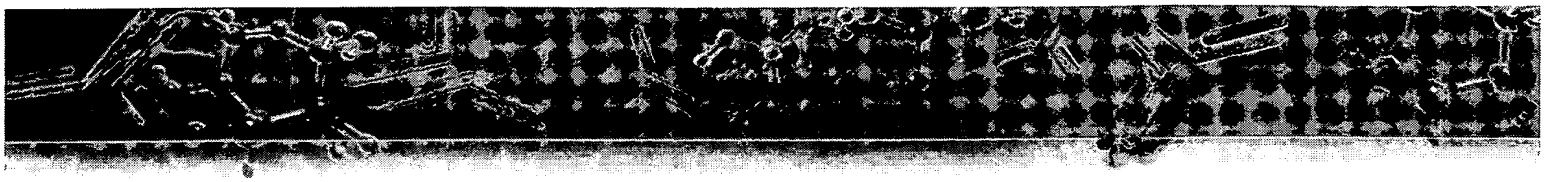
Preparing a Working Stock Solution (General approach)

Make a stock solution that is at a higher concentration than required for the experimental assay by dissolving the peptide in sterile distilled water or sterile dilute acetic acid (0.1%), where applicable. The stock solution peptide can be diluted further with the assay buffer. If the assay buffer is used initially to dissolve the peptide and fails to dissolve, recovery of the peptide, free of nonvolatile salts and/or organic solvents can be a challenge. If the peptide does not dissolve in water or acetic acid, the peptide solution can be lyophilized without any nonvolatile residues. Once the peptide is lyophilized, other stronger solvents can be tried.

Guidelines for Dissolving Several Peptides

A recommended strategy for redissolving sets of peptides containing varying properties is described below. This procedure may result with the final working stock solution of each peptide possessing different volume levels.

1. Add 0.1% acetic acid/water to yield a target concentration of 1-5mg/mL and sonicate the sample.
2. For any insoluble peptides add pure acetic acid to bring the concentration of the acetic acid to 10%(v/v), and sonicate the sample.
3. If peptides are still insoluble, add acetonitrile to 20%(v/v), and sonicate the sample.
4. Lyophilize any remaining insoluble peptides to remove the water, acetic acid and acetonitrile. When the sample is completely dry, add neat DMF or DMSO (dropwise) until the peptide dissolves. Slowly dilute the solution with water to approximately 10%(v/v) DMF or DMSO. If precipitation occurs at any stage during this step, stop adding water and add more DMF or DMSO (dropwise) until the peptide completely dissolves. These peptides may be too insoluble in water to be used at the same concentration of the others in the set.
5. Dilute each solubilized peptide with the most effective solvent to bring the stock solutions to the same peptide concentration. This will simplify working with the peptides in your experimental assay. Further dilutions can be made in the assay buffer. Diluting insoluble peptides with buffer at this step may eliminate the occurrence of precipitation because it is now below its solubility limit.
6. All solutions, except those containing DMF or DMSO, can be lyophilized. This will return the peptide to a suitable state for optimal long-term storage.



Storage Guidelines for Peptide Solutions

The shelf life of peptide solutions is limited. Peptides containing N, Q, C, M and W are unstable when stored in solution. Using sterile buffers (pH 5-6) and freezing the aliquots will prolong the storage life of the peptide. Storage at -20°C or colder is optimal. Avoid repeated freeze-thaw cycles, as this can degrade the peptides.

Peptide Stability and Potential Degradation Pathways

The stability of peptides varies with the amino acid composition. A peptide can degrade if appropriate storage conditions are not used. In addition to the risk of degradation from proteolytic enzymes, other chemical changes can occur. The section below outlines some possible degradation pathways that can arise.

1. Hydrolysis - This is generally a problem in peptides containing Asp (D) in the sequence, which is susceptible to dehydration to form a cyclic imide intermediate. If the sequence contains Asp-Pro (D-P), the acid catalyzed formation of cyclic imide intermediate can result to cleavage of the peptide chain. Similarly, if Asp-Gly (D-G) is present in the sequence, the cyclic intermediate can be hydrolyzed either into the original Asp form, which is harmless, or into a potentially inactive iso-aspartate analog. Eventually, all of the aspartate form can be completely converted into the iso-aspartate analog. To a lesser extent, sequences containing Ser (S) can also form cyclic imide intermediate that can result in cleaving the peptide chain.
2. Deamidation - This base-catalyzed reaction occurs in sequences containing Asn-Gly (N-G) or Gln-Gly (Q-G) and follows a mechanism analogous to the Asp-Gly (D-G) sequence. The deamidation (loss of amine) of the Asn-Gly sequence forms a cyclic imide intermediate that is subsequently hydrolyzed to form the aspartate or iso-asparate analog of Asn. In addition, the cyclic imide intermediate can lead to racemization into D-Asp or D-iso-Asp analogs of Asn, all of which can potentially be inactive forms.
3. Oxidation - The Cys (C) and Met (M) residues are the predominant residues that undergo reversible oxidation. Oxidation of cysteine is accelerated at higher pH, where the thiol is more easily deprotonated and readily forms intra-chain or inter-chain disulfide bonds. Disulfide bonds can be readily reversed by treatment with dithiothreitol (DTT) or tris(2-carboxyethylphosphine) hydrochloride (TCEP). Methionine oxidizes by both chemical and photochemical pathways to form methionine sufoxide and further into methionine sulfone, both of which are almost impossible to reverse.
4. Diketopiperazine and pyroglutamic acid formation - Diketopiperazine formation occurs when Gly (G) is in the third position from the N-terminus, and more readily if Pro (P) or Gly (G) is in position 1 or 2. The reaction involves nucleophilic attack of the N-terminal nitrogen on the amide carbonyl between the second and third amino acid, which leads to the cleavage of the first two amino acids in the form of a diketopiperazine. On the other hand, pyroglutamic acid formation is almost inevitable if Gln (Q) is at the N-terminal position of the sequence. This is an analogous reaction where the N-terminal nitrogen attacks the side chain carbonyl carbon of Gln (Q) to form a deaminated pyroglutamayl peptide analog. This conversion also occurs in peptide containing Asn in the N-terminus, but to a much lesser extent.

Conclusion

The most effective way to prevent or minimize peptide degradation is to store the peptide in lyophilized form at -20°C or preferably at -80°C (if available). If the peptide is in solution, freeze-thaw cycles should be avoided by freezing individual aliquots. Exposure to pH>8 should be avoided. However, if it is necessary to dissolve peptides at pH>8, the solutions should be chilled. Finally, prolonged exposure of lyophilized peptides and solutions (especially at high pH) to atmospheric oxygen should be minimized.